

# The Combined Therapy of Insulin and Zinc Improves Glucose Uptake and Utilization by Testicular Cells in Diabetic Rats

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## Abstract

Glucose uptake by the Sertoli cells of the testes is essential for spermatogenesis. Diabetes mellitus (DM) alters the uptake and utilization of glucose by the testicular cells. This manifests as one of the causes of male subfertility/infertility in diabetes mellitus. With onset of complications even before diagnosis, especially in developing countries, there may be need for therapy beyond the standard therapy. Five normal and twenty diabetic male rats were assigned into groups as follows; normal control had five normal rats with access to food and water only. DM group had five diabetic rats with no treatment. DM and insulin group had five diabetic rats and given subcutaneous insulin twice daily. DM and zinc group had five diabetic rats and given 10mg/kg oral zinc. The final group had five diabetic rats and given both insulin and zinc as above. Treatments in all cases only commenced after fourteen days. Testes were harvested after ten days of treatment for assay. DM decreased both glucose and lactate in the testes. Insulin and zinc improved the uptake of glucose and its utilization as evident in increased production of lactate.

**Keywords:** Insulin, Zinc, Testicular glucose metabolism, Diabetes mellitus, Lactate dehydrogenase, Lactate.

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## INTRODUCTION

Spermatogenesis is a metabolic process that is dependent on glucose (Zysk *et al.*, 1975). Glucose and other metabolites are carried in the blood and will have to cross the blood-testis barrier (BTB) and be taken up by the Sertoli cells (Mruk and Cheng, 2012). This barrier is among the tightest blood-tissue barriers in the body (Setchell *et al.*, 1969). The Sertoli cell (SC) is the most important testicular cell requiring efficient functioning of the BTB (Sharpe *et al.*, 2003). The SCs are 'nurse cells' that nourish and physically support developing germ cells (Mita and Hall, 1982). They are endowed with glucose transporters that help regulate their glucose uptake (Galardo *et al.*, 2008; Oliveira *et al.*, 2011; Rato *et al.*, 2012). The glucose is metabolised to lactate (Jutte *et al.*, 1981; Rato *et al.*, 2012) that is used by the germ cells (Nakamura *et al.*, 1982). The conversion of glucose to lactate requires lactate dehydrogenase (LDH) enzyme (Goldberg, 1985; Goldberg *et al.*, 2010). In vitro studies on cultured insulin-deprived SCs showed altered glucose metabolism (Oliveira *et al.*, 2012).

Diabetes mellitus (DM) is a complex metabolic pathology marked by elevated blood glucose caused by either defective insulin secretion or action. It could be caused by a combination of both (American diabetes association, 2011). It is a disease accompanied by several complications, among which are neuropathy, retinopathy, nephropathy, cardiovascular disorders and infertility (Atkinson and Maclaren, 1994; Melendez-Ramirez *et al.*, 2010). DM impairs male fertility at several levels, one of which is alterations of glucose metabolism in the testes (Alves *et al.*, 2013c). With the prevalence of DM rising, male fertility is likely to become more widespread.

The late diagnosis of DM, especially in developing countries, allows for cellular deterioration that may not be reversed or ameliorated with standard therapy alone. With oxidative stress playing a major role in the pathogenesis of DM, this study therefore sought to establish whether zinc; a known antioxidant ((Stumvoll *et al.*, 2005) and insulin combined therapy will improve testicular glucose metabolism following the onset of DM

## MATERIALS AND METHODS

### Chemicals/Drugs

Streptozotocin (STZ), insulin and zinc gluconate were purchased from Sigma-Aldrich, St. Louis, MO, USA, Novo Nordisk A/S, Denmark and Pharmedic, Vietnam respectively. All other chemicals used were of analytical grade and commercially available.

### Experimental Animals

Twenty five sexually mature male Wistar rats aged 8 to 10 weeks with weights between 150 – 230g were purchased from the animal house of the Anatomy department, University of Benin, Benin City, Nigeria. They were housed in standard cages. Sawdust was used as beddings. They were kept in the Animal House of the Department of Physiology, University of Calabar, Calabar, Nigeria, where they were allowed to acclimatize for 2 weeks before the commencement of experiment. Animals had free access to water and were handled according to approved guidelines of Animal ethics committee of the Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria.

### Study Design

Five normal rats and twenty diabetic rats were randomly assigned to five groups consisting 5 animals each viz: Normal control, Diabetic mellitus (DM) control, Diabetes mellitus (DM) and Insulin, Diabetes mellitus (DM) and Zinc, Diabetes (DM) and Insulin and Zinc.

Animals were fasted overnight and by a single intraperitoneal injection of streptozotocin (STZ) at 60mg/kg, (De Young *et al.*, 2004) DM was induced. 0.1M citrate buffer (pH 4.5) was prepared and used as vehicle. By a prick of the tail vein, blood gotten was checked for fasting glucose 48 hours following DM induction. Fasting blood glucose greater or equal to 250 mg/dL was considered diabetic (Cao *et al.*, 2012). For the next two weeks, All animals had free access to only normal feed and water. Treatments only commenced at the expiration of two weeks. Insulin was administered two times daily by subcutaneous route to animals in the DM and insulin group, at 1 and 4 units in the morning and evening respectively (Pinheiro *et al.*, 2011). 10mg/kg of zinc gluconate was given once daily by oral route to animals in the DM and zinc group (Shidfar *et al.*, 2010). The last group received both insulin and zinc as in the doses and routes described above. Animals were sacrificed and testes harvested for analyses after ten days of treatment.

## METHODS

### Preparation of testicular homogenates

Testes (Right) were homogenized in cold 0.1M phosphate buffer (pH 7.4) using a homogenizer and Teflon pestle. The homogenate was thereafter centrifuged at 3500g for ten (10) minutes. The

supernatants were then assayed for glucose, lactate dehydrogenase enzyme and lactate.

### Measurement of testicular glucose

The measurement of glucose level in the testicular tissue homogenate was done using glucometer. The values were expressed in mg/dL

### Determination of lactate

The enzymatic method was employed to assay for lactate using kit. The principle is based on the conversion of lactate to pyruvate catalysed by LDH. NAD<sup>+</sup> is used as substrate and is reduced to NADH. Lactate concentration is proportional to the amount of NADH that is reduced. The absorbance is determined at 340nm using a spectrophotometer.

### Determination of testicular lactate dehydrogenase (LDH)

Testicular LDH was determined using methods described by Wahlefeld (1983). Sodium lactate and nicotinamide adenine dinucleotide (NAD) were used as substrates. The formation of reduced NAD (NADH) at absorbance of 340 nm was used to determine LDH activity in testis.

### Statistical analysis

Data were analysed using graph pad prism. One-way analysis of variance (ANOVA) followed by Tukey post-hoc test was used and results presented as mean  $\pm$  standard error of mean. P value less than 0.05 ( $p < 0.05$ ) was considered significant.

## RESULTS

### Testicular Glucose

Fig 1 shows that there was a significant decrease ( $P < 0.001$ ) in testicular glucose in DM control ( $49.40 \pm 0.75$  mg/dl), DM + insulin ( $66.60 \pm 1.21$  mg/dl), DM + zinc ( $56.20 \pm 1.59$  mg/dl) and DM + insulin + zinc ( $77.40 \pm 0.98$  mg/dl) groups when compared to the normal control group ( $99.00 \pm 1.70$  mg/dl). When compared to the DM control, testicular glucose increased significantly ( $P < 0.001$ ) in DM + insulin and DM + insulin + zinc groups and in the DM + zinc group ( $P < 0.05$ ). Testicular glucose was significantly decreased ( $P < 0.001$ ) and significantly increased ( $P < 0.001$ ) in the DM + zinc and DM + insulin + zinc groups respectively when compared to the DM + insulin group. When compared to the DM + zinc group, there was a significant increase ( $P < 0.001$ ) in testicular glucose in the DM + insulin + zinc group.

### Testicular lactate dehydrogenase (LDH)

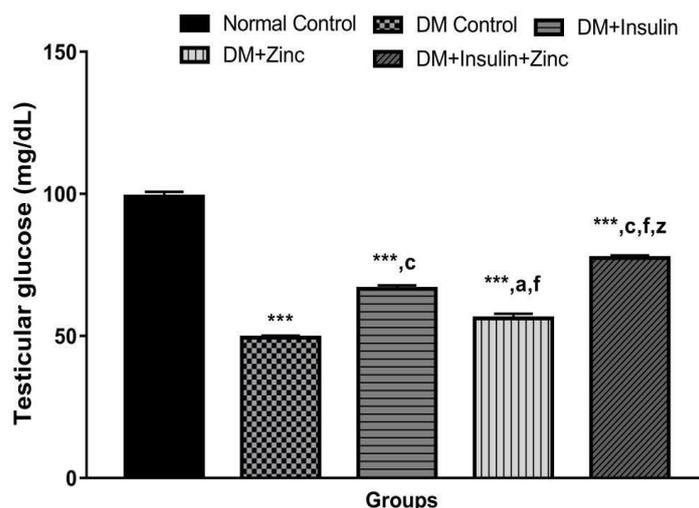
There was a significant increase ( $P < 0.001$ ) in testicular LDH in DM control ( $38.92 \pm 0.48$  U/L), DM + insulin ( $29.58 \pm 0.36$  U/L), DM + zinc ( $27.71 \pm 0.79$  U/L) and DM + insulin + zinc ( $22.77 \pm 0.27$  U/L) groups when compared to the normal control group ( $14.26 \pm 0.27$  U/L). Results further show there was a significant decrease ( $P < 0.001$ ) in testicular LDH in DM

+ insulin, DM + zinc and DM + insulin + zinc groups when compared to the DM control group. Testicular LDH decreased significantly in the DM + insulin + zinc group when compared to DM + insulin ( $P < 0.001$ ) and DM + zinc ( $P < 0.001$ ) groups (Fig 2)

**Testicular lactate**

Result (Fig 3) show there was significant decrease ( $P < 0.001$ ) in testicular lactate in DM control ( $0.24 \pm 0.01$  umol/g), DM + insulin ( $0.54 \pm 0.01$

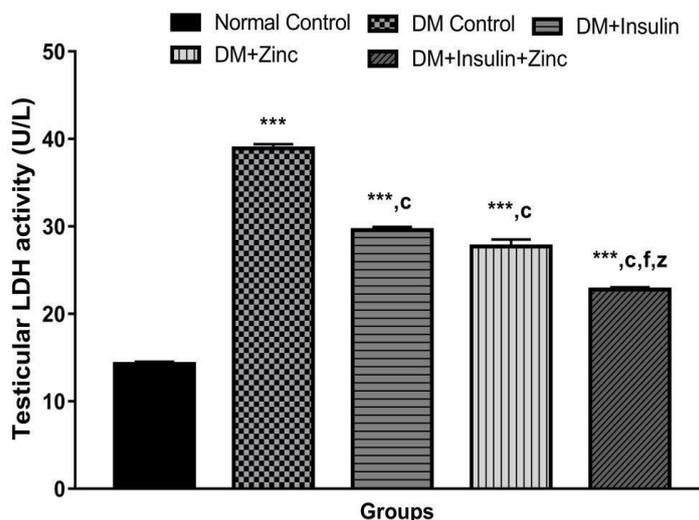
umol/g), DM + zinc ( $0.42 \pm 0.01$  umol/g) and DM + insulin + zinc ( $0.74 \pm 0.02$  umol/g) groups when compared to the normal control group ( $1.15 \pm 0.07$  umol/g). When compared to the DM control, testicular lactate increased significantly ( $P < 0.001$ ) in the DM + insulin, DM + insulin + zinc and DM + zinc ( $P < 0.01$ ) groups. There was a significant increase in testicular lactate in the DM + insulin + zinc group when compared to DM + insulin ( $P < 0.01$ ) and DM + zinc ( $P < 0.001$ ) groups.



**Fig 1: Comparison of testicular glucose concentration between the different groups**

Values are mean  $\pm$  SEM, n = 5.

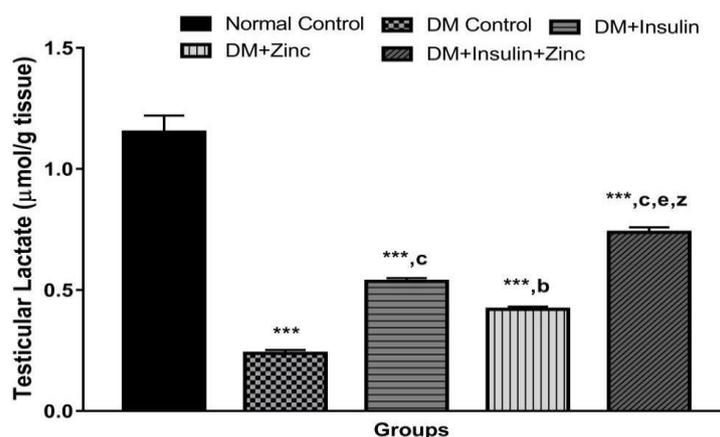
\*\*\* $p < 0.001$  vs Normal control; a =  $p < 0.05$ , c =  $p < 0.001$  vs DM control; f =  $p < 0.001$  vs DM + Insulin; z =  $p < 0.001$  vs DM + Zinc



**Fig 2: Comparison of testicular LDH activity between the different groups**

Values are mean  $\pm$  SEM, n = 5.

\*\*\* $p < 0.001$  vs Normal control; c =  $p < 0.001$  vs DM control; f =  $p < 0.001$  vs DM + Insulin; z =  $p < 0.001$  vs DM + Zinc.



**Fig 3: Comparison of testicular lactate concentration between the different groups**

Values are mean  $\pm$  SEM, n = 5.

\*\*\*p<0.001 vs Normal control; b = p<0.01, c = p<0.001 vs DM control; e = p<0.01 vs DM + Insulin; z = p<0.001 vs DM + Zinc.

## DISCUSSION

Glucose uptake and its subsequent metabolism by testicular cells are essential for normal reproductive function (Alves *et al.*, 2013a). The maintenance of spermatogenesis is dependent on testicular glucose metabolism (Zysk *et al.*, 1975). Glucose, once taken up by the Sertoli cells is converted to lactate by lactate dehydrogenase (LDH) enzyme (Robinson and Fritz, 1981; Rato *et al.*, 2010). Lactate is needed for the nourishment of germ cells (Alves *et al.*, 2013b).

In the present study, there was a decrease in testicular glucose in all the diabetic groups (both treated and untreated) which naturally translated to reduced testicular lactate. DM is associated with insulin deficiency/insufficiency which results in impaired glucose uptake by tissues (Munoz *et al.*, 1996; American Diabetes Association, 2011). This is the probable reason for decreased testicular glucose, which in turn affected lactate production by the sertoli cells. This is in agreement with Oliveira *et al.*, (2012), where glucose uptake by sertoli cells was decreased as was lactate production in insulin-deprived sertoli cells *in vitro*.

Treatments however increased both glucose and lactate. Insulin administration most likely led to increased glucose uptake by the testis eventually resulting in increased lactate, and may also have had a direct impact on lactate production by SCs. Insulin has been reported to increase lactate production (Oonk *et al.*, 1985). Zinc is reported to stimulate insulin production and increase insulin sensitivity (Alkaladi *et al.*, 2014). This is the likely mechanism by which it increased glucose uptake. Acting in synergy, insulin and zinc co-administration provided better results.

As stated earlier, LDH converts glucose to lactate. In this study however, the observed increase in testicular LDH in the diabetic groups, didn't directly

translate to increased lactate levels. It thus appears this increased LDH levels may be due to testicular damage caused by oxidative stress. DM is known to cause oxidative stress (La Vignera *et al.*, 2012). Oxidative stress has been shown to cause an elevation in LDH (Ohta *et al.*, 2009).

## CONCLUSION

Glucose metabolism in the testes is a critical requirement for effective spermatogenesis. Diabetes mellitus alters testicular glucose metabolism. Insulin or zinc ameliorates alterations in testicular glucose metabolism. However, Insulin and zinc co-administration improves testicular glucose metabolism better than either insulin or zinc following diabetes mellitus.

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